ToxCast™ Fact Sheet

A. Purpose of ToxCast

The National Center for Computational Toxicology (NCCT) of the Office and Research and Development of the U.S. Environmental Protection Agency (EPA) is undertaking the ToxCast™ research program to develop cost-effective innovative approaches to prioritize a large number of chemicals in a short period of time for toxicological testing. Using data from state-of-the-art high throughput screening (HTS) bioassays developed in the pharmaceutical industry, ToxCast is building computational models to forecast the potential human toxicity of chemicals. These hazard predictions should provide the Agency's regulatory programs with science-based information helpful in prioritizing chemicals for more detailed toxicological evaluations, and therefore lead to using animal tests more efficiently.

B. Description of ToxCast Program

ToxCast is designed to test the hypothesis that multi-dimensional evaluation of chemical properties and effects across a broad spectrum of information domains (e.g., molecular, cellular, and organ responses) will provide data that will be predictive of toxicity. EPA thinks that, with sufficient information on a range of chemicals, EPA can identify distinctive patterns of results in HTS bioassays, so called “bioactivity signatures,” that are strongly correlated with specific types of toxic effects observed in traditional animal toxicity testing. The ToxCast predictive bioactivity signatures will be based upon physical-chemical properties, predicted biological activities based on existing structure-activity models, biochemical properties from high throughput screening assays, cell-based phenotypic assays, genomic analyses of cells, and responses in non-mammalian model organisms. These bioactivity signatures will be defined and evaluated by their ability to predict outcomes from existing mammalian toxicity testing.

ToxCast is now profiling the responses of over 300 chemicals (mainly pesticides and other select chemicals), chosen because of our toxicological understanding from existing traditional animal test data and their intended activities as biological agents. More than 400 HTS and high-content assays are being provided by nine extramural contracts capable of handling up to 10,000 chemicals over a five-year period. An Interagency Agreement has also been established with the National Chemical Genomics Center (NCGC) of NIH to provide access to the novel technologies developed by the NIH Molecular Libraries Initiative. An initial investment of over $6M has been made in the nine contracts generating HTS data, with results expected by the end of 2007. The second phase of ToxCast is projected to involve up to 1000 additional chemicals representing broader chemical structure and use classes, in order to evaluate the predictive bioactivity signatures developed in Phase I.

HTS refers to a system that rapidly and efficiently tests large numbers of chemicals for bioactivity, typically utilizing robotics and automation applied to molecular biology and cellular assays. The over 400 ToxCast HTS assays will provide information, quickly and in a cost-efficient manner, on the potential impact of chemicals on numerous biological pathways critical for the function of systems such as the heart, lungs, brain or reproductive organs. The goal of ToxCast is to use computational

methods to build models to predict potential for toxicity of environmental chemicals. It is estimated there are more than several thousand environmental chemicals from EPA programs that could be prioritized for further testing. For example, antimicrobials, inert ingredients in pesticide products, new and existing industrial chemicals, and drinking water contaminant candidates generally have limited toxicity data available for hazard and risk assessments. ToxCast is being developed to potentially provide a means to efficiently and quantitatively prioritize these chemicals for additional toxicological evaluation based on computational models using chemical descriptors and biological activity profiling.

This five-year effort will be divided into three phases. In Phase I, as a proof-of-concept, ToxCast will examine more than 300 chemicals, in over 400 different HTS bioassays, to create predictive bioactivity signatures based on the known toxicity of the 300 chemicals. The Phase I chemicals are primarily pesticide active ingredients that have been extensively evaluated by traditional mammalian toxicity testing, and hence have known properties representative of a number of phenotypic outcomes (e.g., carcinogenicity; and developmental, reproductive and neural toxicity). Phase II will focus on the confirmation and expansion of ToxCast predictive signatures, generating HTS data on over 1000 additional chemicals. In Phase III, ToxCast will be expanded to the thousands of environmental chemicals, delivering an affordable, science-based system for categorizing chemicals. As the ToxCast database grows, so will confidence in predicted toxicity and potential mechanisms of action useful in refining and reducing the use of animals in toxicity testing. Phase I results are anticipated by the summer of 2008. To ensure transparency and collaboration, ToxCast™ data will be freely available at PubChem, a National Library of Medicine public database that stores information about the biological activities of small molecules.

Results from the ToxCast HTS assays will provide, for the first time, a comprehensive and detailed overview of the potential impact of environmental chemicals upon key cellular activities. The assays range from characterizing the interactions of chemicals with proteins that regulate and maintain proper cell function, to measuring the response of whole cells, to studying chemical effects in a model organism, for up to 10,000 environmental chemicals. The ToxCast HTS assays are being run through nine contracts awarded in April 2007 to ACEA Biosciences, Inc. (San Diego, CA); Attagene, Inc. (Morrisville, NC); BioSeek Inc. (Burlingame, CA); Cellumen, Inc. (Pittsburgh, PA); Compound Focus, Inc. (South San Francisco, CA); Expression Analysis, Inc. (Durham, NC); In Vitro ADMET Laboratories (IVAL), LLC. (Rockville, MD); NovaScreen Biosciences Corp. (Hanover, MD); and Phylonix Pharmaceuticals, Inc. (Cambridge, MA).

In order to utilize the millions of datapoints stemming from ToxCast, it is necessary to have electronic information available in formats that will allow sophisticated data mining and analysis. ToxRefDB (Toxicology Reference Database) provides a relational database of standard toxicity test results for pesticides, making it possible to link toxicity information with the HTS and genomic data of ToxCast. The potential for ToxCast to use HTS and genomic data for environmental chemical hazard assessment, screening, and prioritization requires initial anchoring of these data to reference toxicological test information. Toxicity data have been produced for thousands of environmental chemicals and, at EPA, these data have been used primarily in hazard characterization and risk assessment of individual chemicals. In

order to extend the use of this vast store of information, ToxRefDB captures toxicological endpoints, critical effects and relevant dose-response data, primarily from EPA Pesticides Program evaluations, in a relational database using a standardized data field structure and vocabulary. The creation and population of ToxRefDB has been a collaborative effort between ORD/NCCT and OPP, and initially ToxRefDB is being populated with data from OPP for pesticidal active chemicals. Comparable toxicity data from other Agency databases (e.g., HPVIS), the National Toxicology Program, and other sources are also being captured in ToxRefDB for non-pesticidal chemicals. ToxRefDB currently provides the ability to cluster and group chemicals based on toxicological outcomes specific to study type, target organ, or effect categories. In addition, ToxRefDB will facilitate ranking of chemicals by relative potency based on specific endpoints, or grouping of chemicals based on mode or mechanism of action. Thus, ToxRefDB will provide the essential interpretive context for linking ToxCast HTS and genomic data to toxicity endpoints.

In order to integrate the relational environment of ToxRefDB with associated chemical structure information, and tools for searching and categorization that will guide development of predictive HTS bioactivity profiles and genomic signatures, a more comprehensive data management system is being developed by NCCT. ACToR (Aggregated Computational Toxicology Resource) will manage the large-scale sets of ToxCast assay data, and is comprised of several independent data repositories, tied together through links to a common database of chemical structures and properties. The main databases cover chemical information, biochemical (HTS) and cell-based assays, detailed in vivo toxicology data (ToxRefDB) , experimental design information, genomics data (mainly microarray), and reference information on genes and pathways. ACToR is collecting information from multiple sources both within and external to the EPA. Users will be able to access data through the web, initially on the EPA Intranet. The first use of ACToR will be to provide a repository and context for data from the ToxCast program's in vitro biochemical and genomics assays.

C. Relationship of ToxCast to NAS Report

The National Research Council of the National Academy of Sciences (NAS) provided EPA a report in June 2007 entitled "Toxicity Testing in the Twenty-first Century: A Vision and a Strategy." The report's overall objective is to foster a transformative paradigm shift in toxicology based largely on the use of in vitro systems that will (1) provide broad coverage of chemicals, chemical mixtures, outcomes, and lifestages, (2) reduce the cost and time of testing, (3) use fewer animals and cause minimal suffering in the animals used, and (4) develop a more robust scientific base for assessing health effects of environmental agents. This vision is highly consistent with the strategic directions and research activities of EPA. Even before the EPA commissioned this report, it was taking steps to incorporate modern biological and computational tools into the evaluation of chemicals hazards and risks, and ToxCast is EPA's major effort to predict hazard, identify key toxicity pathways, and intelligently prioritize chemicals for targeted, hypothesis-driven animal testing. The endorsement of this approach by the NRC provides further assurance that the Agency's ToxCast program is on the right track.

D. Relationship of ToxCast to EPA Strategic Needs

ToxCast is a departure from mainstream toxicology, and will require regulatory acceptance by the Agency and stakeholders alike for successful applications in chemical prioritization. Developing ToxCast within EPA, an organization that uses cutting edge tools at the bench level, and is responsible for protecting human health and the environment, will facilitate regulatory acceptance as the science unfolds. The NCCT is working across the Agency, with other Federal agencies, and with stakeholder groups to develop this help advance the transformation called for by the NRC.

ToxCast holds the promise to complement and expand existing chemical screening approaches by efficiently and quantitatively prioritizing EPA-relevant chemicals based on computational models using chemical descriptors and biological activity profiling. Armed with this science-based information, EPA programs can further prioritize chemicals for more detailed evaluations, including using animal tests more efficiently.

E. Current Collaborations

The EPA Chemical Prioritization Community of Practice (CPCP) was formed in December 2005 to advance research into the utility of computational chemistry, high-throughput screening and various toxicogenomic technologies for Agency use. The goal of the CPCP is to provide a venue for stakeholder information sharing and discussion related to these technologies and for interpretation in order to categorize chemicals and predict toxicity. The CPCP is chaired by the NCCT and meets monthly by teleconference. It has a membership of over 100 individuals from 20 public and private sector organizations.

As a result of collaborations established through the CPCP, NCCT has entered into an Interagency Agreement (IAG) with the National Institute of Health's Chemical Genomics Center (NCGC) to profile biological activity of a large collection of environmental chemicals. The objective of the 5-year IAG is to generate data to support the ToxCast chemical prioritization project being developed by NCCT. As of June 2007, NCGC is running assays on 1,408 chemicals selected by the EPA, using the industrial-scale HTS technologies. Testing will employ both biochemical and cellular assays and be conducted in state-of-the-art, quantitative-HTS format providing both potency and efficacy information against a large number of biological targets. An initial focus of the IAG will be on functional assays for nuclear receptors. Results of the testing are being made available through deposition into PubChem, a National Library of Medicine public database storing information about the biological activities of small molecules.

Internationally, the Organization of Economic Cooperation and Development (OECD) has supported a project proposal developed jointly by the NCCT and the Office of Pollution Prevention and Toxic Substances (OPPTS) to promote international cooperation and research on application of new molecular based approaches for the prioritization and screening of environmental chemicals for potential toxicity. The objective of the "Molecular Screening for Characterizing Individual Chemicals and Chemical Categories Project" project is to establish a strategy for rationally and

economically prioritizing chemicals for further evaluation, based on molecular properties and categories linked to potential toxicity. This objective directly builds on the goals of the ToxCast program, and the needs of EPA relevant to various chemical programs. Recognizing the need for international acceptance and harmonization of molecular screening tools, the NCCT and OPPTS approached OECD about facilitating such an activity. The project was formally accepted by the joint OECD/International Programme on Chemical Safety Advisory Group on Toxicogenomics in January 2007, and a workshop was held in May 2007 to initiate collaborative efforts. It is likely that several countries and companies will continue to actively participate. In the 2008 – 2009 timeframe, further development of partnering arrangements, infrastructure, and information sharing will occur. Also during this timeframe, a specific list of chemicals and methodologies could be agreed upon for the OECD-coordinated effort. The OECD Molecular Screening Project represents a valuable opportunity for the Agency to link the ToxCast program to international research trying to develop solutions to the increasing demand for chemical testing, and provide science-based prioritization for the toxicity testing of environmental chemicals.

The EPA is very interested in continuing to engage other organizations in collaborative research arrangements in which we share experiences, efforts, and best practices relevant to ToxCast, HTS screening, and chemical prioritization. In order to facilitate this, the NCCT expects to issue a solicitation, through the Agency's Office of Science Policy, for Cooperative Research and Development Agreement (CRADA) partners to join in the ToxCast effort. Please reply to Kathleen Graham at graham.kathleen@epa.gov or 202-564-2678 if you are interested in partnering on this CRADA. For more information on the ToxCast CRADA please see the following website: http://www.epa.gov/osp/ftta.htm

For more information:

- ToxCast™ Program http://www.epa.gov/ncct/toxcast/
  [Dix et al. 2007. The ToxCast program for prioritizing toxicity testing of environmental chemicals. Toxicol Sci. 95(1):5-12]
- National Center for Computational Toxicology http://www.epa.gov/ncct/
- Chemical Prioritization Community of Practice www.epa.gov/ncct/practice_community/category_priority.html
- National Library of Medicine public database, PubChem: http://pubchem.ncbi.nlm.nih.gov/

ToxCast™ Frequently Asked Questions (FAQ):

1. What is ToxCast™?

ToxCast™ is a new research program sponsored by EPA's Office of Research and Development (ORD) that uses high throughput screening (HTS) tools that allows for the collection of a large amount of experimental data in a short period of time on a wide variety of biochemical, genetic, and molecular assays. The results of these HTS assays will be correlated with findings from traditional animal toxicity testing in order to identify patterns of biological responses (also called "signatures") that are predictive of adverse outcomes such as cancer, birth defects, reproductive dysfunction or nervous system impairment.

2. Why is EPA conducting this research?

Success of the ToxCast research program could lead to more effective and efficient use of EPA resources in protecting human health and the environment, by providing a science-based approach to screen a large number of environmental chemicals, and to prioritize and guide more rational animal testing approach based on the chemicals' toxicological potential and expected environmental exposure.

3. Who is paying for ToxCast?

EPA is investing over $6M in Phase I of ToxCast. Continuing research in Phase II of ToxCast will involve a larger number and more diverse set of chemicals than Phase I, and EPA is seeking research partners to help facilitate the Phase II research program.

4. How long will the ToxCast research last?

The ToxCast research program is being conducted in two phases. Phase I biological activity results from HTS assays for approximately 300 chemicals are expected in 2008. Phase II of ToxCast, that will test the predictivity of HTS signatures identified in Phase I, will occur in the 2008-2010 timeframe. A Phase III regulatory application of ToxCast would follow successful completion of the research phases.

5. How did EPA select the chemicals in ToxCast?

Phase I of ToxCast is now profiling the response of over 300 chemicals (mainly pesticides and other select chemicals), chosen because EPA has extensive toxicity data, derived from traditional animal testing, on each of the chemicals. The list of candidate chemicals includes most of the food-use pesticide active ingredients, 16 High Production Volume (HPV) chemicals, 11 of which are part of the HPV Challenge, and 57 of the 73 chemicals proposed for the initial Tier 1 screening of the Endocrine Disruptor Screening Program (EDSP). The identity of chemicals involved in ToxCast Phase I will be available at www.epa.gov/ncct/toxcast.

6. Is there information available on the specific assays & specific protocols each contactor will conduct?

An initial description of most of the ToxCast Phase I assays is available at www.epa.gov/ncct/toxcast. Protocols are available for some of the assays but not all. More descriptive information on the assays will be provided as the HTS data are generated and made public.

7. Will all chemicals be run in every assay? If not, what specifics chemicals will be run in each assay? How will these be determined?

With the exception of the zebra fish assays, the chemicals in Phase I of ToxCast will be run in all the other assays.

8. Will these assays be run in accordance with GLPs?

In the research phases of ToxCast, the assays will not be run according to GLP. However, rigorous QA/QC requirements are being met by each of the EPA contractors through Quality Management Plans that include detailed Standard Operating Procedures and proven assay reliability. When ToxCast is brought into a regulatory framework, it is anticipated that these assays will be conducted under GLP.

9. Will these assays be validated before they are used? In accordance with ICCVAM?

All the ToxCast assays have proven reproducibility based on reliability, sensitivity, and specificity using negative and positive controls. Performance during ToxCast will continue to be monitored, and evaluated through CRADA and OECD partnerships. Since ToxCast assays are not being presented as alternatives to currently required animal testing, there are currently no plans for ICCVAM validation.

10. How were the High Throughput Screening (HTS) assays evaluated for reproducibility and reliability?

The Phase I ToxCast HTS assays are all commercially available assays being provided by nine extramural contracts, with demonstrated reproducibility based on reliability, sensitivity, and specificity using negative and positive controls. A description and list of the assays involved in ToxCast Phase I will be available at www.epa.gov/ncct/toxcast

11. How will the predictability of the HTS assays be evaluated in ToxCast Phases I and II?

A pattern of biological responses (i.e., "signatures") identified in Phase I HTS assays of ToxCast will be evaluated for sensitivity and specificity through cross-verification and testing with blinded chemical subsets of Phase I. Phase II will incorporate chemicals with toxicity data for further verification of predicted toxicity outcomes based on Phase I signatures.

12. What further performance criteria will be used to determine which assays from Phase I are necessary for reliable predictors and thus should continue on to Phases II or III?

Multiple predictive models will be tested with the wide range of HTS data from Phase I of ToxCast. The combination of model(s) and data that produce the most sensitive and reasonably specific predictions of toxicity outcomes will be selected. This approach may require different models for different toxicity predictions, as necessary, for attaining the goal of minimizing false negatives.

13. Presumably, some if not many, of the ToxCast™ assays will be proprietary. Does this create any issues for use of this information by EPA or others?

The ToxCast program is designed to help in prioritization of chemicals for further testing. Though some of the ToxCast assays are proprietary all of them are commercially available. Furthermore, the molecular or biological targets and testing conditions for these assays are well-defined and measurable through a variety of approaches.

14. What is Toxicology Reference Database (ToxRefDB)?

ToxRefDB is a relational database capturing the toxicity results (e.g., animal chronic, subchronic, reproductive, and developmental studies) in a searchable format. The goal of ToxRefDB is to provide scientists with a tool to correlate the results of traditional toxicological data with the results of high throughput biological assays.

15. Will the data from the Toxicology Reference Database (ToxRefDB), and results from ToxCast Phase I and II be publicly available? If so, when?

Agency scientists are currently in consultation with the Office of General Counsel (OGC) regarding under which conditions ToxRefDB could be made available to the public.

16. Is a prototype of the ToxRef Database available for review?

The schema for the database is available at www.epa.gov/ncct/toxcast

However, this schema does not contain the data currently being entered into ToxRefDB

17. Will all the data from the pesticide DER be included in the ToxRef database? For example, there may be several studies in one or more species or strains for each endpoint. Will each study be entered into the ToxRef database?

The ToxRefDB is designed to be a repository for the toxicological data pertaining to each chemical. ToxRefDB will capture summary data from all the relevant DERs on all toxicologically significant effects (e.g., effects at LOAEL, magnitude of effects, etc.)

18. Will Data Evaluation Records (DER) for the pesticide toxicity studies that are used to populate ToxRefDB be publicly available? If so, when?

There are no plans to release DERs. Should anyone wish to see specific DERs, they can be requested from the US EPA under the Freedom of Information Act (FOIA).

19. Will ToxCast have any effect on Tier1 testing in the Endocrine Disruptors Screening Program (EDSP), and specifically, would evaluation of a chemical in ToxCast Phases I or II result in a chemical being selected for EDSP Tier 1 testing or in a waiver of some EDSP screens?

Since ToxCast is still in the R&D phase, it is currently not viewed as suitable for priority setting or as a replacement for any assay that will comprise the EDSP's Tier 1 battery. After sufficient data have been generated and analyzed, the Agency will determine how best to utilize ToxCast, including whether there is potential for its use within the EDSP.

20. Would a response in ToxCast Phase I or II trigger a data call-in, FQPA factor, FIFRA 6a2, or pesticides' labeling action?

Phase I and II of ToxCast™ are the research phases of the program, and thus are not intended to be used in regulatory decisions or in risk assessments. ToxCast™ will not be used as a regulatory tool until it has been adequately peer reviewed and its proposed uses have undergone public comment.

21. Would a response in ToxCast™ Phase I or II trigger a TSCA 8(e) submission?

The data generated by ORD, their contractors, and other collaborators which are made available to ORD would be considered known to the Administrator and thus not subject to 8(e) notification If any concerns beyond this arise, then the specific scenarios should be submitted to EPA's OPPT for consideration.

22. How will ToxCast™ be brought into a regulatory or voluntary context once Phase II is completed? and when?

A careful stepwise approach will be taken to ensure appropriate use and interpretation of ToxCast data by regulatory programs. When Phase II is completed and there is scientific consensus that ToxCast™ provides reliable predictions for purposes of screening and prioritization, the next step will be to move this tool carefully into the regulatory programs by first conducting extensive outreach and education activities, allowing sufficient time for comments and input from stakeholders and international partners, and peer review on the regulatory applications.

23. How can other organizations establish collaborative efforts with the ToxCast™ program?

The EPA is interested in finding partners for a Cooperative Research and Development Agreement (CRADA) to further develop, evaluate and expand its ToxCast program for predicting the toxicity of environmental chemicals. In particular, EPA seeks CRADA partners interested in contributing to the ToxCast™ research program by generating additional HTS data with Phase I chemicals, or supporting generation of HTS data with Phase II chemicals. Potential partners include, but are not limited to, non-govermental organizations, private sector companies, academic institutions, and companies with expertise in predictive toxicology. More information on the ToxCast CRADA is available at http://www.epa.gov/osp/ftta.htm, and http://www.epa.gov/osp/ftta/ToxCast.pdf.